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Comparative Study of Nd:YAG Laser Angioplasty at 1.06 μm, 1.32 μm, and 1.44 μm Wavelengths: Decreased Vascular Spasm and Early Mortality With 1.44 μm Laser Ablation

James Bauer, MSc, Xiu-Yan Jiang, MD, Yue Wen, MD, Weidong Yan, MD, Erbin Dai, MD, Li-ying Liu, MD, John Tulip, PhD, and Alexandra R. Lucas, MD

Department of Electrical Engineering, University of Alberta, Edmonton, Alberta (J.B., J.T.); and Division of Cardiology, Department of Medicine, University of Alberta, Edmonton, Alberta, Canada, T6G 2B7 (X.-Y.J., Y.W., W.Y., E.D., L.-Y.L., A.R.L.)

Background and Objective: **Although laser angioplasty has been demonstrated to be effective for the treatment of long, complex coronary arterial atherosclerotic stenoses, there is an associated risk of acute arterial spasm, dissection, and perforation as well as a significant restenosis rate. It has been postulated that the use of lasers emitting at wavelengths designed for radiation absorption by water would decrease local tissue trauma.**

Study Design/Materials and Methods: **We have examined the use of a Nd:YAG laser designed to emit at 1.44 μm, an absorption peak for water, and compared the results of laser ablation at 1.06 μm, 1.32 μm, and 1.44 μm wavelengths. Nd:YAG laser angioplasty was performed in the abdominal aorta of White Leghorn roosters. Acute and chronic vascular trauma was assessed by contrast angiography and histological analysis.**

Results: **There was a significant decrease in early mortality with 1.44 μm laser ablation. This decreased mortality after 1.44 μm ablation was associated with a decrease in vascular spasm, perforation, and thermal damage. Atherosclerotic plaque development at follow up was decreased with 1.44 μm ablation but this was not significant.**

Conclusion: **1.44 μm laser ablation decreases early vascular trauma and mortality and may decrease subsequent atherosclerotic plaque development.**

Key words: angioplasty, atherosclerosis, Nd:YAG, vascular injury, water absorption

INTRODUCTION

There is a considerable risk of arterial trauma during laser angioplasty; acute arterial spasm, intimal dissection, and thrombosis have been detected in 2–20% of cases [1–9]. Restenosis is also a significant problem occurring in 30–50% of cases after interventional therapy, regardless of the device used [10–13]. This has limited the use of laser angioplasty particularly due to the fact that there is only a clear improvement in acute and chronic results after excimer or pulsed laser angioplasty in chronic total coronary arterial occlusion [9]. Many other new devices have also been developed (mechanical atherectomy and stent implantation) with the express intention of reducing the amount of arterial trauma associated with recannalization of a stenotic artery and

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Address reprint requests to Dr. Alexandra Lucas, Division of Cardiology, Department of Medicine, University of Alberta Hospitals, Edmonton, Alberta, Canada, T6G-2B7.

decreasing the degree of local recurrent growth of the obstructing plaque [14–18]. Numerous lasers as well as laser angioplasty probes and catheters have also been devised in an attempt to address this problem, but none of these devices has succeeded in decreasing the incidence of acute and chronic arterial occlusion [1–9].

Nd:YAG laser angioplasty has been associated with thermal trauma to the arterial wall that extends the degree of damage beyond the obstructing atherosclerotic lesion targeted for treatment, e.g., recannalization [19–23]. The healing response in the arterial wall has been found to be similar for pulsed and continuous wave laser ablation although there have been preliminary reports of a decrease in platelet activation at sites of thermally induced arterial injury with continuous wave laser ablation [22]. Within the scope of this study, laser ablation or photovaporization is primarily a thermal process [24]. The extent of thermal damage to tissue is dependent on the volume of tissue which absorbs the incident energy. When the applied energy is absorbed in a sufficiently small volume, the associated temperature rise within the tissue will exceed 100°C and local photovaporization will occur. If however, the energy is absorbed in a relatively large volume, the temperature increase will not be enough to cause vaporization, but there may still be detrimental thermal damage to the tissue. The parameter which relates the depth, and hence volume, over which the energy is absorbed is the absorption coefficient. The absorption coefficient of water, a dominant constituent of biological tissue, is shown in Figure 1. Several laser wavelengths of interest are indicated in the same figure. Based on this, it would be expected that the use of a Nd:YAG laser designed to operate at 1.44 μm, a wavelength strongly absorbed by water, has the potential to be an effective laser ablation device while limiting the degree of thermal damage to adjacent tissue [24,25].

The two aims of this study were 1) to assess the effects of incidental damage to normal arterial intima during laser ablation and 2) to compare the effects of the Multi-Nd:YAG laser emitting at 1.06 μm and 1.32 μm, standard Nd:YAG emission wavelengths, and 1.44 μm, a wavelength in the range where light absorption by water is enhanced. In order to examine the effects of this wavelength selectable Nd:YAG laser on arterial intima, the early and late effects of laser angioplasty on normal arterial wall were studied in roosters over a 2–12 week period after laser ablation (Multi-YAG laser designed by Dr. J. Tulip, Department of Electrical Engineering, University of Alberta, Edmonton, Alberta, Canada).

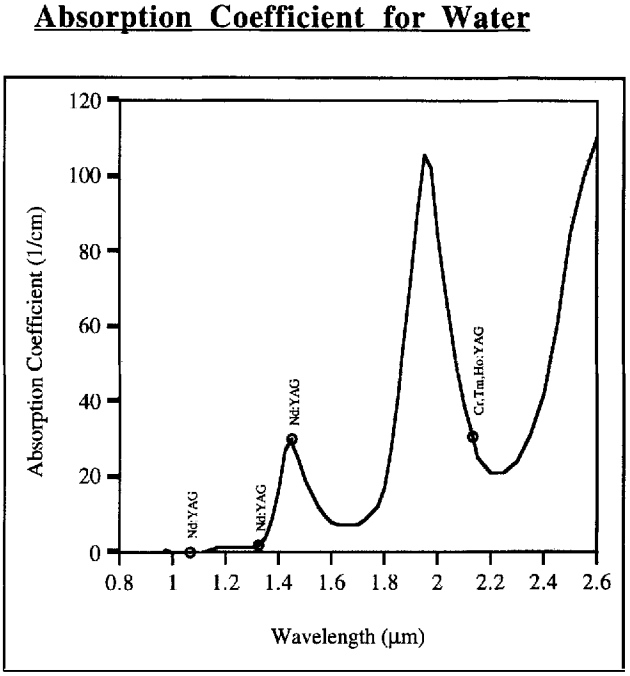


Fig. 1. Water absorption-maximum absorption peaks are observed at 1.44 μm and 2.0 μm. The absorption is equivalent for the Nd:YAG emitting at 1.44 μm and the holmium YAG at 2.1 μm.

MATERIALS AND METHODS

Rooster Model

Thirty-two White Leghorn roosters (age 1–2 years, 2.1–3.0 kg) were used as an avian model for atherosclerotic plaque development after arterial injury induced by Nd:YAG laser ablation at 1.06 μm, 1.32 μm, and 1.44 μm wavelengths. This model has been used and developed in our laboratory for investigation into restenosis as well as plaque development [26,27]. Under general inhalation anesthetic, Hallothane titrated to effect and administered through a hood attachment designed for use in roosters, a 5 French Cook sheath was advanced retrograde into the femoral artery via cut down. Four hundred Units of heparin was given intra-arterially at the beginning of each procedure in order to reduce catheter induced thrombosis in the aortic lumen. Baseline contrast angiograms were obtained prior to and after laser ablation and at follow up. Roosters were sacrificed at selected time intervals between 2–12 weeks for histological assessment after follow-up angiography.

Roosters were maintained on normal poultry diet throughout the study. The roosters were carefully monitored postoperatively in the lab animal services surgical recovery unit until stable. All animal surgery and subsequent care conformed to both local University animal services and National laboratory animal ethics guidelines.

Laser Ablation

A preliminary assessment of the optimal energy level to be used for laser ablation at each wavelength (1.06 μm, 1.32 μm, and 1.44 μm) was performed in human aorta specimens post mortem in vitro and in 6 roosters in vivo. Eighteen human aorta sections were examined after 1.06 μm ablation at energies ranging from 0.75 J (7.5 W, 0.1 sec) to 16 J (10 W, 1.6 sec); 20 sections were examined after 1.32 μm ablation at energies ranging from 0.5 J (5 W, 0.1 sec) to 16 J (10 W, 1.6 sec); and 29 sections were examined after ablation at 1.44 μm at energies ranging from 0.15 J (1.5 W, 0.1 sec) to 16 J (10 W, 1.6 sec). The human aortic specimens ranged from normal arterial histology to moderate, fibrotic atherosclerosis.

In addition, a preliminary in vivo assessment in roosters was used to determine the energy to be used for these studies in vivo. The energy levels were chosen to create a cut extending to the depth of the media but which prevented excess vascular trauma or perforation. Two roosters had 1.06 μm ablation at a range of energies from 1.5 J (7.5 W, 0.2 sec) to 15 J (10 W, 1.5 sec); three roosters had 1.32 μm ablation at energies ranging from 4 J (5 W, 0.8 sec) to 15 J (10 W, 1.5 sec); and one rooster had 1.44 μ ablation at energies ranging from 0.3 J (1.5 W, 0.2 sec) to 15 J (1.5 W, 1.0 sec). The roosters used for this preliminary assessment of optimal energy levels were sacrificed immediately after laser surgery in order to assess the amount of laser induced trauma. The degree of damage and any associated perforations were noted by contrast angiography, histological analysis and by simple visual inspection. Each site of laser induced ablation was marked with Mrs. Stewart's bluing and the aorta was subsequently fixed in neutral buffered formalin for later histological analysis.

Based on histological analysis and simple visual inspection, the energy levels chosen for subsequent laser ablations was 5 J (5 W, 1 sec) for 1.06 μm ablation, 5 J (5 W, 1 sec) for 1.32 μm ablation, and 0.6 J (1.5 W, 0.4 sec) for 1.44 μm. The energy at 1.44 μm wavelength ablation was less than the other energy levels chosen for 1.06 μm and 1.32 μm ablation because the higher tissue absorption at 1.44 μm wavelength results in perforation at higher energy levels. Thirty two roosters had laser ablation of the aorta at either 1.06 μm, 1.32 μm, or 1.44 μm wavelengths: 10 roosters had 1.06 μm laser ablation, 14 roosters had 1.32 μm laser ablation, and 8 roosters had 1.44 μm laser ablation. After base line angiography, a 400 μm fiber (3M EOTec™ power core fiber, 3M Fiber Optic Products, West Haven, CT) was inserted through the sheath into the distal abdominal aorta and then advanced to the thoracic aorta under fluoroscopic control. Laser ablation was performed with the laser catheter in contact with the intimal surface, roughly perpendicular to the intimal surface. Each rooster had 8–12 sites of laser ablation in the thoracic and abdominal aorta while under general anesthetic.

Contrast Aortography

Angiograms were recorded in a single projection, posteroanterior, during contrast injection on Kodak EG6 film with a Quanta 111 screen, using an Orthopedic Equipment Company (OEC, Ultrasound division, Salt Lake City, UT) Varian Model 901 MF10 C arm image intensifier and portable fluoroscopy system. Radiopaque contrast (7–10 ml) was injected retrograde into the distal iliac artery through the femoral arterial sheath. A metal marker was recorded to allow for standardization of aortic diameter measurements. The diameters of the normal abdominal aorta and detected stenoses, either in the aorta or in aortic branches, were measured by electronic micrometer or caliper (Mitutoyo Model SR44 Digimatic, Tokyo, Japan).

Diameter stenosis was measured and recorded routinely on all angiograms. The areas measured on all angiograms were the area just proximal to the cranial mesenteric artery, the abdominal aorta midway from the cranial mesenteric artery to the iliac bifurcation, and the area just proximal to the iliac bifurcation [28]. The arterial diameter stenosis was calculated by dividing the diameter measured at each site by the aortic diameter recorded from 1 cm proximal to the superior mesenteric artery. Any other visible sites of stenosis or dissection were also measured in the thoracic and abdominal aorta, the major branches of the abdominal aorta, and the non instrumented iliac artery when available. The decrease in the aortic diameter normally observed on comparison of the distal abdominal aorta with the thoracic aorta was also examined.

Morphometric Histological Analysis

After sacrifice with euthanyl (3.0–4.0 ml/kg, 240 mg pentobarbitol, and 0.20 ml propylene glycol per ml) (MTC Pharmaceuticals, Cambridge, Ontario), the aorta was fixed in neutral buffered formalin and embedded in paraffin for sectioning as has been previously described [26,27]. Sections were stained with hematoxylin and eosin for analysis of intimal hyperplasia and atherosclerotic plaque development after laser ablation. Aortic arterial specimens harvested after laser ablation, were also assessed for the depth of laser penetration and the degree of local thermal damage and charring adjacent to the sites of laser ablation. Seventy-one specimens were processed for histological examination both after acute damage and after follow up (60 had morphometric analysis); 11/71 after 1.06 μm ablation, 39/71 after 1.32 μm ablation, and 21/71 after 1.44 μm ablation. Histological specimens taken immediately after laser ablation in vivo were assessed separately from the second chronic rooster study in vivo. The human aortic post-mortem sections were similarly fixed and stained immediately after laser ablation.

Each histological slide was assessed for changes in the amount of laser induced thermal damage by measuring the depth and width of thermal damage (ablation depth, eosinophilia, vacuolization, confetti, fusion, and charring) associated with laser ablation by ocular micrometer and by morphometric analysis. Atherosclerotic plaque development and associated changes in fatty, fibrous, calcific, and thrombotic changes associated with plaque growth were also assessed by morphometric analysis using a Nikon drawing tube attachment connected to a Labophot 2 Nikon microscope (Nikon Canada, Ltd., British Colombia). Plaque area as well as laser ablation depth and width of damage were then measured by means of a Summagraphics Model ADIBI Bit Pad Plus tablet (Summagraphics Corporation, Austin, TX) pad connected to a Macintosh IIcx computer and Mac Measure program.

Statistics

Angiographic measurements of arterial diameter and the morphometric analysis of plaque area and thickness were each compared with the laser ablation wavelength used in each group of experimental rooster protocols. The results were compared by chi square analysis and Student's *t*-test. The presence or absence of early and late mortality, complications associated with laser ablation (spasm, dissection, and perforation), stenoses greater than 20–30%, and plaque area as well as plaque thickness were compared by chi-square analysis and Student's *t*-test. Correlation between the presence of a measured plaque area greater than 0.5 mm^2, 1.0 mm^2, and 1.5 mm^2 and fatty plaque content greater than 0.01 mm^2, 0.05 mm^2, and 0.1 mm^2 with the laser ablation wavelength were assessed by chi-square analysis. Plaque area and thickness, fatty, fibrous, and calcific plaque content as well as the presence of thrombus associated with plaque and the depth and width of laser ablation and associated thermal damage were also assessed both by chi-square analysis, ANOVA analysis, and Student's *t*-test.

RESULTS

Assessment of the Energy Levels to be Used for Laser Ablation

A preliminary study to assess the energy levels to be used at each laser wavelength was performed using post mortem human aorta specimens in vitro in addition to using rooster aorta in vivo.

For human post-mortem aorta (both histologically normal and containing atherosclerotic plaque), a significant difference in the efficacy of ablation was observed with the 1.44 μm wavelength on comparison with ablation at 1.06 μm and 1.32 μm (Table 1, Fig. 2). A markedly greater degree of ablation at 1.44μm (Fig. 2A) on comparison with 1.06 μm and 1.32 μm (Fig. 2B) at energies over the entire range applied was measured by histological analysis. If however, the ablation depth and width after 1.44 μm irradiation at energies less than or equal to 1 J (Fig. 2C) is compared with ablation at 1.06 μm and 1.32 μm at greater than 1 J (Fig. 2B) there is no significant difference in either the depth or width of laser induced arterial injury. These results indicate that the depth of ablation for 1.44 μm at less than 1 J is comparable to ablation at greater than 1 J for 1.06 μm and 1.32 μm laser energy. There was visible perforation and marked thermal trauma for ablation at 1.44 μm at energies greater than 5 J and at energies greater than 10 J for 1.06 μm, and 1.32 μm ablation both on histological analysis and visual inspection.

In the rooster model, there was visible perforation at energy levels greater than 10 J for 1.06 μm wavelength YAG laser ablation and for

TABLE 1. Histologic Analysis of Ablation Energy on Human Aortic Arterial Specimens

Wavelength (μm)	Energy range (J)	Width ablation (mm)	Depth ablation (mm)
1.06	0.75–16	0.947 ± 0.123	1.280 ± 0.196
1.32	0.5–16	0.895 ± 0.107	0.990 ± 0.170
1.44	0.15–16	1.343 ± 0.081[a]	1.524 ± 0.132[b]
1.06	≥1.0	0.938 ± 0.134	1.215 ± 0.229
1.32	≥1.0	0.928 ± 0.130	1.043 ± 0.220
1.44	≤1.0	1.100 ± 0.153[c]	1.411 ± 0.275[c]

[a] $P < 0.007$ for 1.44 vs.1.06, $p < 0.004$ for 1.44 vs 1.32
[b] P = NS for 1.44 vs 1.06, $p < 0.03$ for 1.44 vs 1.32
[c] P = NS for 1.44 vs 1.06, p = NS for 1.44 vs 1.32

energy levels in excess of 7.5 J for 1.32 μm laser ablation. Perforation was seen with energies above 0.9 J for 1.44 μm laser ablation. At ablation energy levels of 5 J for 1.06 μm, 5 J for 1.32 μm (Fig. 3A), and 0.6 J for 1.44 μm laser ablation there was effective arterial intimal ablation but no detectable perforation (Fig. 3B). This choice of energy levels for arterial ablation studies was later born out by the finding that although there was increased lateral spread of thermal injury associated with laser ablation at 1.06 μm and 1.32 μm ablation both in the in vitro study involving ablation in human aorta specimens and in the in vivo study in rooster aorta, there was a similar depth of laser penetration into the arterial wall at all three wavelengths of ablation.

Mortality

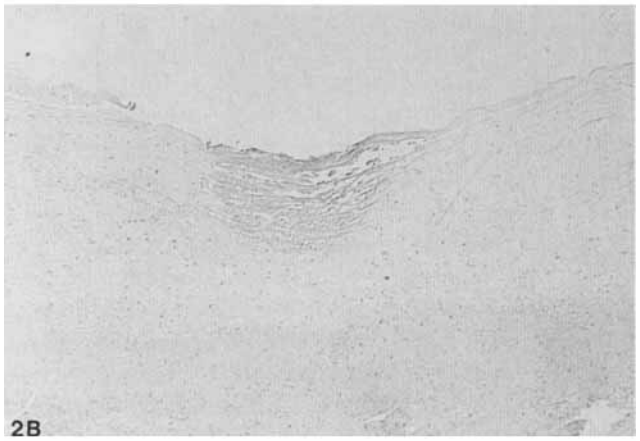
An increase in early mortality was detected with 1.06 μm and 1.32 μm laser ablation when compared to 1.44 μm ablation (Fig. 4); 5/14 roosters that had 1.32 μm ablation and 3/10 rosters that had 1.06 μm ablation died within 2 weeks after laser ablation. In contrast, 1/8 roosters that had 1.44 μm ablation died within 2 weeks after ablation ($P < 0.0015$ by chi-square analysis). The late mortality, unexpected mortality at greater than 2 weeks, was also increased after 1.06 μm and 1.32 μm ablation on comparison with 1.44 μm ablation (borderline significant at $P = 0.031$ by chi-square analysis).

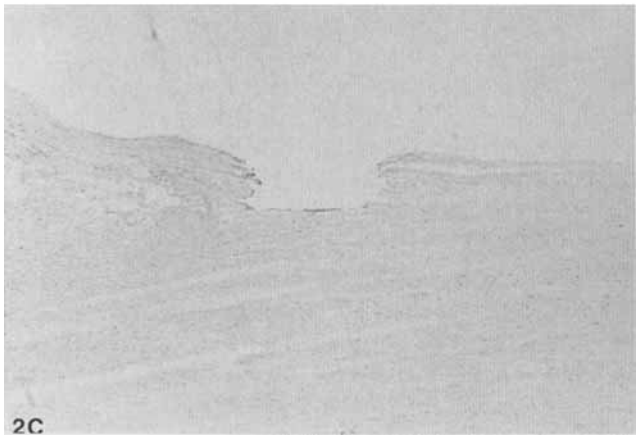
Contrast Aortography

There was a noticeable increase in laser induced trauma (vascular spasm, dissection, and perforation) immediately after ablation at 1.06 μm and 1.32 μm (Fig. 5A); again in contrast to 1.44 μm ablation (Fig. 5B) where the frequency of spasm, dissection, and perforation was markedly reduced. Arterial spasm was detectable in 3/10 (30%) of angiograms recorded immediately after 1.06 μm ablation, 5/14 (35.7%) of specimens after 1.32 μm ablation, and 0/10 (0%) of specimens after 1.44 μm ablation, $P < 0.0005$ by chi-square analysis. The range of the measured percent stenosis was 40.8–72.0% immediately after 1.06 μm ablation, 42.2–60.6% immediately after 1.32 μm ablation, and 0–70.8% immediately after 1.44 μm ablation. There was also a significant increase in the rate of observed dissection and perforation after 1.06 μm and 1.32 μm ablation. There were 2/10 dissections and 1/10 perforations after 1.06 μm ablation, 3/14 dissections and 2/14 perforations after 1.32 μm ablation, and 1/8 dissections and 0/8 perforations after 1.44 μm ablation (chi square analysis: $P < 0.001$ for dissections and $P < 0.0009$ for perforations observed after 1.06 μm and 1.32 μm ablation).

Evidence of vascular damage, acute arterial spasm, and dissection was decreased both immediately after laser ablation and at follow up with the use of 1.44 μm laser ablation when compared to either 1.06 μm or 1.32 μm ablation (Fig. 5). The mean percent diameter stenosis immediately after laser surgery for all angiograms recorded was 24.8 ± 3.8% after 1.06 μm ablation, 33.1 ± 4.4% after 1.32 μm ablation, and 23.1 ± 3.3% after 1.44 μm ablation. The mean percent diameter stenosis at follow up was 28.1 ± 9.9% after 1.06 μm ablation, 19.0 ± 2.6% after 1.32 μm ablation, and 18.7 ± 3.2% after 1.44 μm ablation. Although there was a trend toward decreased mean percent stenosis after 1.44 μm ablation particularly on comparison with 1.06 μm ablation, these values did not reach statistical significance by Student's *t*-test.

When the angiograms were analyzed and compared only in groups divided into those angiograms recorded in roosters that died suddenly (early mortality), and those that had follow-up at





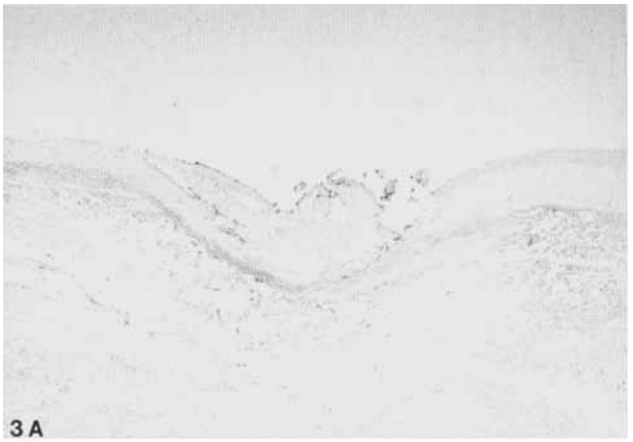


Fig. 2. Laser ablation of human aorta necropsy specimens (less than 72 hours old) at 1.06 μm, 1.32 μm, and 1.44 μm wavelengths. The depth of laser ablation is markedly greater at 1.44 μm wavelength ablation (**A**) than at 1.06 μm and 1.32 μm wavelength ablation (**B**) at energies greater than 5 W ($P < 0.0001$ by ANOVA). The depth of laser ablation at 1.44 μm wavelength ablation at less than 5 W energy is comparable, actually greater, in the observed depth of ablation (**C**) but has decreased width of laser thermal damage (P = NS, by ANOVA). All sections were stained with hematoxylin and eosin (magnification, ×260).

Fig. 3. Hematoxylin and eosin stained histological sections taken from sites of laser ablation in vivo in rooster aorta. **A:** Sections taken at a site of 1.32 μm ablation demonstrating the depth of laser ablation and the surrounding fusion, confetti, and charring created by laser ablation seen in some specimens immediately after 1.32 μm and 1.06 μm ablation (magnification, ×260). **B:** A site or 1.44 μm ablation which demonstrates that the ablation depth was similar but that there was minimal surrounding thermal damage evident (magnification, ×260).

the previously selected time intervals, there was a clear trend to a decrease in the measurable percent stenosis after 1.44 μm ablation. The mean percent stenosis measured in the early mortality cases was 37.7 ± 6.4% after 1.06 μm ablation, 46.3 ± 7.6% after 1.32 μm ablation (mean value for 1.06 and 1.32 data combined was 37.7 ± 6.4% stenosis), and 30.6 ± 5.7% after 1.44 μm ablation

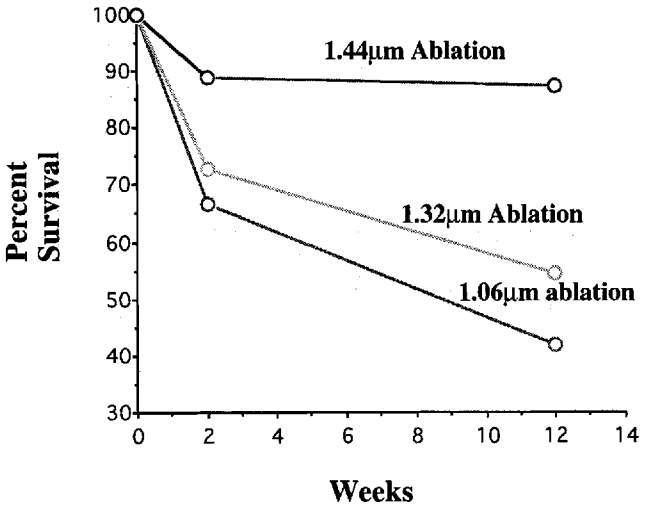


Fig. 4. Percent survival after laser angioplasty at 1.06 μm, 1.32 μm, and 1.44 μm ablation. There is a clear increase in survival after 1.44 μm ablation when compared to 1.06 μm and 1.32 μm ablation.

(Fig. 5C). When the angiographic data was also compared for roosters that survived to follow up the mean percent stenosis measured after 1.06 μm ablation was 28.1 ± 4.8%, 22.5 ± 2.1% stenosis for 1.32 μm ablation, and 19.7 ± 4.2% stenosis for 1.44 μm ablation (P = NS by Student's t-test, Fig. 5D).

An increase in the number of arterial stenoses was observed immediately post ablation after 1.06 μm and 1.32 μm ablation on comparison with 1.44 μm ablation in roosters that died early secondary to laser associated complications. There were 5/10 (50%) stenoses measured at greater than 30% after 1.06 μm ablation, 12/20 (60%) after 1.32 μm ablation, and 2/4 (50%) after 1.44 μm ablation ($P < 0.0001$ by chi square analysis for >30% stenoses for a comparison of 1.44 μm ablation with 1.06 μm and 1.32 μm ablation). At long term follow up (2–12 weeks after ablation in roosters that survived to follow up), there was a significant decrease in the frequency of detectable stenoses of greater than 30% after ablation with either 1.06 μm or 1.32 μm on comparison with 1.44 μm ablation. There were 5/19 (26%) angiograms with greater than 30% stenosis at follow-up after 1.44 μm ablation and 8/35 (23%) angiograms with greater than 30% stenosis after 1.06 μm and 1.32 μm ablation ($P < 0.0037$ by chi-square analysis for a comparison of 1.44 μm ablation with 1.06 μm and 1.32 μm ablation).

Prior to ablation in either the angiograms recorded from the roosters that died early (less than 2 weeks) or the roosters that died at follow-up (2–12 weeks), there was no significant difference in the number of sites with greater than 30% stenosis. For early mortality roosters there were 0/4 with greater than 30% stenosis after 1.44 μm ablation and 0/24 with greater than 30% stenosis after 1.06 μm and 1.32 μm ablation. For late follow-up angiograms in roosters that survived more than 2 weeks there were 2/47 (4%) angiograms that had greater than 30% stenosis prior to ablation with 1.06 μm and 1.32 μm ablation and there were 1/18 (5%) angiograms with greater than 30% stenosis prior to 1.44 μm ablation (P = NS by chi-square analysis).

This angiographic follow-up data indicated that in spite of the improved mortality as well as the decreased incidence of early spasm, dissection, or perforation there remains a continued propensity to recurrent plaque development. Thus the early increase in laser related complications, spasm, dissection, and perforation with 1.06 μm and 1.32 μm ablation was associated with an increase in early mortality when compared to 1.44 μm ablation. This increased early complication rate with 1.06 μm and 1.32 μm ablation was not however associated with a significant later increase in the rate of detectable atherosclerotic stenoses as detected on angiography in the roosters that survived to follow-up (2–12 weeks).

Histology

There was a significant increase in the width of measurable laser thermal damage (eosinophilia, connective tissue fusion, confetti, vacuolization, and charring) after 1.06 μm and 1.32 μm ablation on comparison with 1.44 μm ablation. Of the 60 histological specimens examined by morphometric analysis, 40/60 specimens had visible evidence of laser induced ablation; 10/40 specimens with laser ablation sites were from 1.06 μm ablation artery specimens, 27/40 were from 1.32 μm ablation specimens, and 4/40 were from 1.44 μm ablation specimens. The mean width of visible laser induced thermal damage was 0.222 ± 0.037 mm for 1.06 μm ablation, 0.494 ± 0.147 mm for 1.32 μm ablation, and 0.047 ± 0.014 for 1.44 μm ablation (Figs. 3, 6). This represents a 10 fold decrease in detectable laser thermal damage extending lateral to the site of ablation (P = 0.0137 for 1.06 μm and 1.32 μm ablation on comparison with 1.44 μm ablation by Student's t-test). There was no measurable difference in the actual depth of laser ablation as measured by morphometric analysis after ablation at

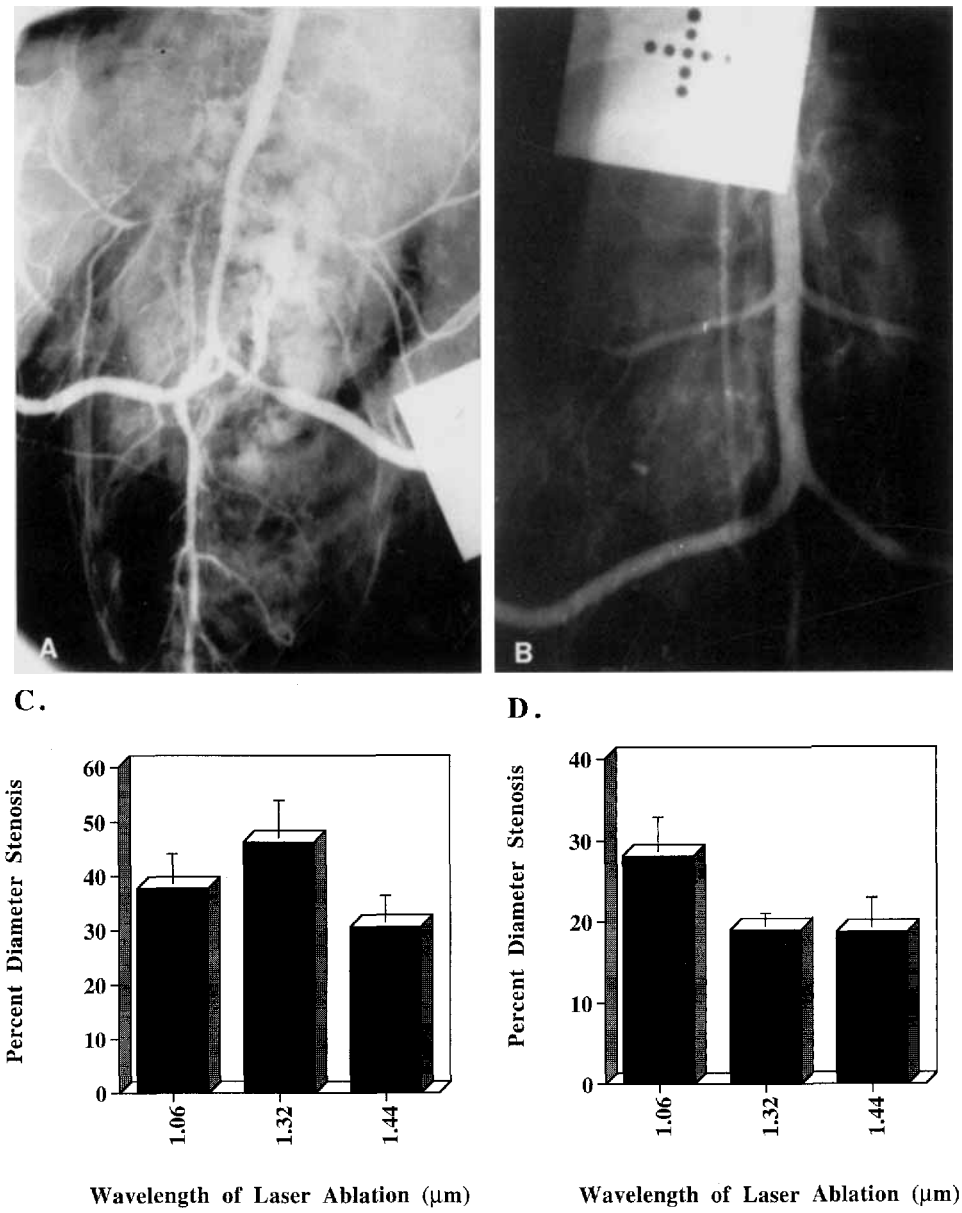


Fig. 5. Contrast angiograms demonstrating early effects of laser ablation on the rooster aorta. **A:** 1.32 μm ablation: Angiogram recorded immediately after 1.32 μm ablation. There is significant associated spasm and haziness suggestive of dissection in the distal abdominal aorta. This rooster died within 48 hours of laser ablation. **B:** 1.44 μm ablation: Angiogram recorded immediately after 1.44 μm ablation. There is minimal evidence of vascular trauma after 1.44 μm ablation. **C:** Bar graph demonstrating a decrease in the diameter stenosis detectable immediately after 1.44 μm laser ablation on comparison with 1.06 μm and 1.32 μm ablation. **D:** Bar graph demonstrating that there is a trend toward a decrease in the arterial diameter after 1.44 μm and 1.32 μm laser ablation at 2–12 weeks follow up after laser ablation on comparison with 1.06 μm ablation.

any of the three wavelengths. The mean depth of laser ablation was 0.154 ± 0.057 mm for 1.06 μm ablation, 0.57 ± 0.142 mm after 1.32 μm ablation, and 0.509 ± 0.244 mm after 1.44 μm ablation (P = 0.9377, by Student's t-test). In fact the mean depth of laser ablation was actually somewhat greater after 1.44 μm ablation than after 1.06 μm ablation (P = 0.2839, NS). Ablation depth was comparable for 1.44 μm laser ablation and the two shorter Nd:YAG laser wavelengths while the extent of thermal damage detectable as lateral spread of injury was about 1/10 that observed at the shorter wavelengths. Visible charring with eosinophilia, vacuolization, and eosinophilia was

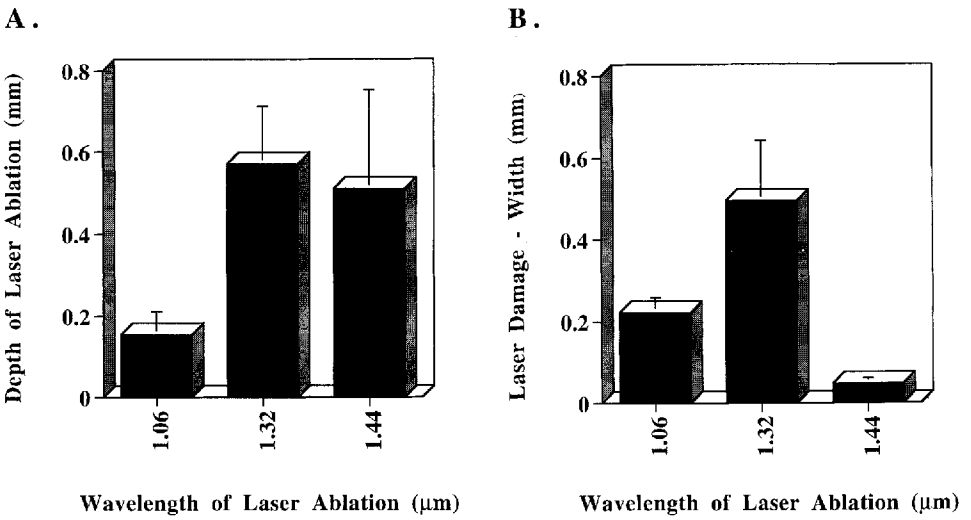


Fig. 6. Bar graphs illustrating the mean depth of laser ablation and the lateral spread of thermal damage after laser ablation at each wavelength used for laser ablation. **A:** Mean ± standard error of ablation depth after laser angioplasty at 1.06 μm, 1.32 μm, and 1.44 μm. **B:** Mean ± standard error of the width of thermal damage after ablation at 1.06 μm, 1.32 μm, and 1.44 μm.

seen at follow-up and was detected in increased frequency after sudden unexpected death, particularly after laser ablation at 1.06 μm and 1.32 μm. The ratio of thermal damage to actual ablated depth produced at 1.44 μm is somewhat smaller than that which has been previously observed [24]. One explanation may be the cooling effect resulting from the flow of blood past the treatment site.

There was a decrease in the mean plaque area measured by morphometric analysis on histological specimens at follow up after 1.44 μm laser ablation when compared to 1.06 μm and 1.32 μm ablation; the mean plaque area was 1.887 mm^2 ± 1.442, 3.049 mm^2 ± 0.964, 1.084 ± 0.238 mm^2 for 1.06 μm, 1.32 μm, and 1.44 μm ablation respectively (Fig. 7). There was also an associated decrease in the thickness of developing plaque after 1.44 μm ablation. This difference was not significant by Student's *t*-test but did demonstrate a significant decrease by chi-square analysis for plaque area greater than 1.5 mm^2 ($P < 0.0001$). However, there was a greater range of plaque area values measured after 1.06 μm and 1.32 μm ablation than after 1.44 μm ablation. The range of measured values for plaque area was 0–20.167 mm^2 for 1.06 μm, 0–16.931 mm^2 for 1.32 μm, and 0–3.229 mm^2 for 1.44 μm ablation. When tested for a significant difference for plaque area greater than 1.0 mm^2 or 0.3 mm^2 there was actually an increased frequency of moderate plaque area in all the specimens examined after 1.44 μm ablation than after 1.06 μm or 1.32 μm ablation. After 1.06 μm ablation, 14% of specimens had measured plaque area greater than 1.5 mm^2 area, 21% had measured plaque area greater than 1.0 mm^2, and 29% had measured plaque area greater than 0.3 mm^2. After 1.32 μm ablation 35% of specimens had plaque area greater than 1.5 mm^2, 35% had plaque area greater than 1.0 mm^2, and 59% had plaque area greater than 0.3 mm^2. For 1.44 μm ablation in contrast, 19% of specimens had plaque areas that were measured as greater than 1.5 mm^2 but 44% had plaque area greater than 1.0 mm^2 and 81% had plaque area measured at greater than 0.3 mm^2. There would appear to be therefore an increase in overall moderate plaque buildup in all specimens after 1.44 μm ablation whereas after 1.06 μm and 1.32 μm ablation there was greater variability in the presence of plaque development with a tendency to development of very large measurable plaques at follow-up.

Plaque composition also differed with decreases in the amount of detectable calcified plaque after 1.06 μm and 1.32 μm ablation. The mean calcified area was measured at 0.043 ± 0.023 mm^2 for 1.44 μm ablation vs. 0 calcific plaque detected after 1.06 μm and 1.32 μm ablation ($P <$ 0.0001 by chi square analysis for presence or absence of calcific plaque content). There was an overall increase in the measurable fatty plaque area after 1.44 μm ablation, 6% greater than

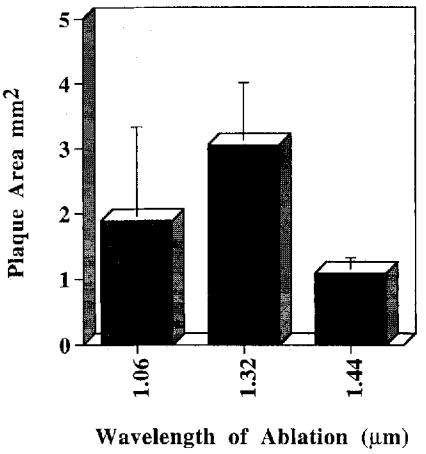


Fig. 7. Bar graph illustrating the morphometric analysis of mean ± standard error of plaque area measured at 2–12 weeks follow-up after laser ablation at 1.06 μm, 1.32 μm, and 1.44 μm ablation. There is a decrease in plaque area after 1.44 μm ablation on comparison with 1.06 μm and 1.32 μm ablation.

0.1 mm^2 fatty area, 38% greater than 0.05 mm^2 fat area, and 63% greater than 0.01 mm^2 fat area. After 1.06 μm and 1.32 μm ablation there were 7% and 26% greater than 0.1 mm^2 fat area, 14% and 28% greater than 0.05 mm^2 fat area, and 29% and 45% greater than 0.01 mm^2 fatty areas, respectively ($P < 0.0001$). There was also a decrease in thrombotic plaque area after 1.44 μm ablation on comparison with 1.06 μm and 1.32 μm ablation. There were no detectable thrombotic areas after 1.44 μm ablation whereas there were 1/13 thrombotic areas after 1.06 μm ablation and 1/29 thrombotic plaque areas after 1.32 μm ablation ($P < 0.0001$ by chi-square analysis for the presence or absence of thrombotic plaque content) (Fig. 8).

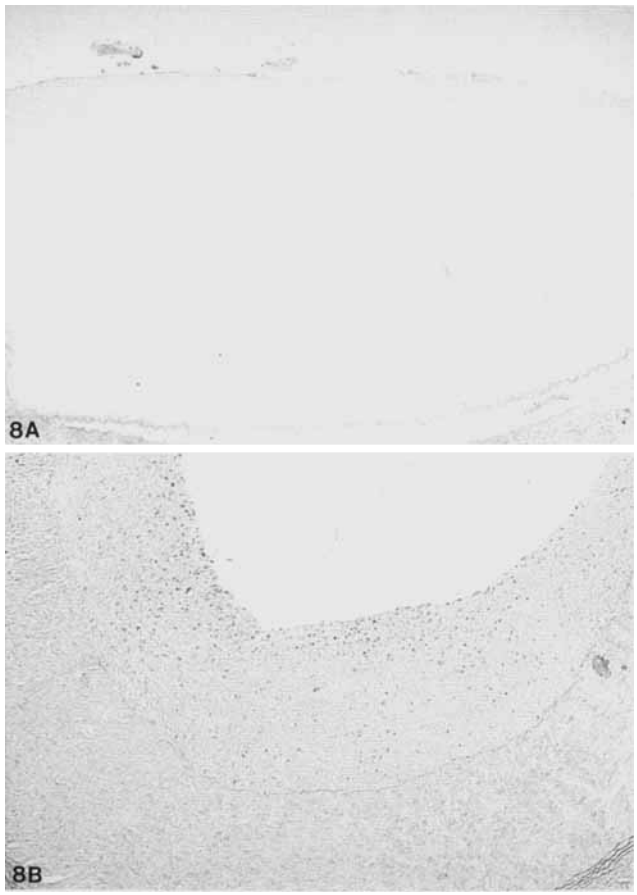


Fig. 8. Hematoxylin and eosin stained sections of aorta at follow-up 2–12 weeks after laser ablation. **A:** Section demonstrating fibrous plaque formation after 1.32 μm ablation at 12 weeks follow-up (magnification, ×260). **B:** Aortic arterial section with evidence of fibrous plaque development and intimal hyperplasia after 1.44 μm ablation (magnification, ×260).

DISCUSSION

This study was undertaken to examine the acute and chronic effects of ablation of normal aortic wall using an Nd:YAG laser operating at 1.06 μm, 1.32 μm, and 1.44 μm. We have demonstrated that there is significant arterial trauma produced in the normal arterial wall and that this laser induced trauma is associated with the later development of atheromatous plaque. Further, the degree of injury to the normal arterial wall is not clearly correlated with the amount of subsequent plaque development detectable in treated animals.

Several different avian models have been developed for the investigation of atherosclerotic arterial disease; White Carneau and Show Racer pigeons, restricted ovulators that lack the LDL/VLDL receptor, Bronze Breasted turkeys, and quail [29–33]. We have examined several interventional devices (stents, excimer lasers, balloon angioplasty, and atherectomy) in White Leghorn roosters. Our results have indicated that there is a good correlation between prior interventional therapy and the subsequent development of fibrous plaque using this rooster model of atherosclerosis. Atherosclerotic lesions in cholesterol fed roosters have been reported to range from simple fatty plaque to fibrous intimal hyperplasia as well as complex calcified and ulcerated atheromatous lesions. Avian models do however differ from mammals in that the erythrocytes and platelets are nucleated. Direct extrapolation on a molecular level may well create discrepancies. However, on a simple histological level, the atherosclerotic lesions have been reported by many investigators to

closely resemble human atherosclerotic plaque. The study was limited by the fact that only normal roosters were used; this work does not necessarily reflect the process that might occur in an artery that already has atherosclerotic plaque. The roosters used in this study were on a normal poultry diet and had not had any prior manipulations or surgery designed to produce atherosclerotic lesions.

The difference in tissue response to 1.06 μm and 1.32 μm radiation was not statistically significant. Applied energy at 1.44 μm was approximately one third of that used at 1.06 μm and 1.32 μm but the depth of ablation was comparable. With 1.44 μm ablation the lateral extent of thermal damage was about one tenth of that observed for shorter wavelength ablation.

This is consistent with the observation that ablation resulting from 1.44 μm laser irradiation resulted in decreased early arterial injury as well as a decrease in early evidence of vascular spasm and perforation. Similarly, there was a decrease in early mortality after laser ablation at 1.44 μm when compared to ablation at 1.06 μm and 1.32 μm. This was identified with a general decrease in the extent of laser induced thermal damage at the longer wavelength. However, there was no significant change in the degree of subsequent plaque development after 1.06 μm, 1.32 μm, and 1.44 μm ablation when observed at follow up times of 2–12 weeks. There was a trend to a reduction in the frequency of observed large accumulations of atherosclerotic plaque following ablation at 1.44 μm while it appeared that there was an increase in the occurrence of small to moderate atherosclerotic lesions.

This study also indicated that injury associated with damage to the normal arterial wall in rooster aorta may play a significant role in the subsequent development of atherosclerotic lesions. In the process of cutting through an atherosclerotic stenosis it would appear likely that the adjacent tissue may be damaged and provide a nidus for the later development of an atheromatous area.

We were unable in this study, to establish the extent of mechanical trauma that was introduced during the procedure that may have contributed to vasospasm or perforation. However, the same laser fibers, catheters, and surgical techniques were utilized for ablations performed with each of the three wavelengths. This should have resulted in the proper controls for comparison and avoided the issue of uncertainty due to changes in any variables associated with mechanical injury.

Laser angioplasty has not been noted to improve the restenosis rate except with long diffuse lesions or total arterial occlusions. There is still a high associated risk of acute arterial perforation and damage. The damage associated with Nd:YAG has generally been characterized by the extent of thermal injury adjacent to the area of ablation. This study indicates that while thermal injury is indeed associated with a high risk of early arterial damage particularly at the 1.06 μm and 1.32 μm wavelengths, there was no overall increase in the later development of atherosclerotic lesion or plaque following ablation with any of the Nd:YAG laser wavelengths used in this study.

There has been recent interest in the use of the Holmium laser for angioplasty. The absorption coefficient of water, and hence tissue, at the 2.1 μm wavelength produced by the Holmium YAG laser (Cr,Tm,Ho:YAG) is comparable to that at 1.44 μm. Therefore, it should be expected that tissue effects produced by these two lasers should be similar. Past studies have concentrated on the energy threshold for ablation of plaque with the Holmium laser and small scale chronic studies. One study has detected a decrease in dissection, spasm, and restenosis after holmium laser angioplasty on comparison with excimer laser angioplasty [34]. Further studies may involve the direct comparison of the Holmium laser with the 1.44 μm Nd:YAG laser based on the decreased arterial trauma and associated early mortality observed in this study.

No prior study has carefully examined the efficacy of the various Nd:YAG laser emission wavelengths as was done in this study. On direct comparison between the selected Nd:YAG wavelengths we have detected a decrease in acute arterial trauma with the use of 1.44 μm irradiation which would suggest that this is an improved wavelength for laser ablation.

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